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Identical Twin Discordance for the Brachmann-de Lange Syndrome Revisited

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The only known twin pair evidently discordantly affected for the BDLS (Brachmann-de Lange syndrome) and who had been considered monozygotic (MZ) based on blood analysis remained a problem because biological zygosity determination needed further typing. In this report we review the clinical findings of this pair of twins at the age of 20. The use of DNA fingerprinting with three multilocus probes, F10, DNF24, and 33.6, allowed us to present evidence of monozygosity with a high degree of certainty. The significance of this confirmation of discordance in determining the cause of BDLS is discussed. Intensive comparative genomic studies of the discordant twin sisters may be useful to unravel the molecular genetics of this enigmatic pattern of malformation.

KEY WORDS: Brachmann-de Lange syndrome, monozygotic twins, DNA fingerprinting

INTRODUCTION

The Brachmann-de Lange syndrome (BDLS) is characterized by mental retardation, microbrachycephaly, synophris, hirsutism, and limb abnormalities including micromelia or phocomelia, in association with prenatal and postnatal growth retardation [Jackson et al., 1993]. Most of the cases of the syndrome are sporadic and although it is suspected that most represent a new autosomal dominant mutation [McKusick, 1995], a genetic cause has not been definitely proven yet. Thus, like in other circumstances in which genetic information is incomplete, considerable interest surrounded the reports of twin pairs with the BDLS.

We could find published descriptions of four sets of monozygotic (MZ) or like-sex twins concordant for BDLS [Opitz et al., 1965; Choo and Bianchi, 1965; Stewart, cited by Stevenson and Scott, 1976 and Opitz, 1985; and Watson, 1979]. These severely affected concordant MZ twins supported the notion of a single gene etiology for BDLS. Discordant, dizygotic twinning were reported by Stevenson and Scott [1976], by Kumar et al. [1985], and by Greenberg and Robinson [1989]. The only supposedly MZ discordant pair described was published in 1976 by two of us [Carakushansky and Berthier, 1976]. At that time monozygosity was inferred on the basis of concordance at the ABO, Rh, MNSs, P, Kell, Lu, Fya, Jk, Xg, transferrin, and haptoglobin loci. In an editorial comment on BDLS, Opitz [1985] urged that more stringent genetic testing be performed on these twins to document more completely their genetic status. In the present report we review the clinical findings of these discordantly affected girls and present strong evidence of monozygosity based on DNA fingerprinting.

CLINICAL REPORT

The clinical aspects of these patients at an early age and the family history were already described elsewhere [Carakushansky and Berthier, 1976]. We reexamined the twins at the age of twenty. The affected twin was very small and short with a weight of 20.7 kg and a height of 123 cm. She had microcephaly (44.7 cm) and a low frontal hairline, synophris, "penciled" eyebrows, short nose, lateral nasal ridge, and crescent shaped mouth (Fig. 1). The ears were apparently low-set and posteriorly angulated. She also had pectus excavatum and complete absence of breast development. Cardiological findings were normal. She had marked diffuse hirsutism with fine black hair on her back and arms. Pubic hair was present in scant amount. There was micromelia, short thumbs with proximal implantation, flexed fingers, and bilateral clinodactyly of fifth fingers with middle phalangeal hypoplasia and single flexion crease. Gross motor development was significantly delayed and was below that of a 1 year old. Fine

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Fig. 1. The twins at age 20. The affected twin presents an absolutely typical clinical picture of the BDLS.

motor function was not adequately testable because the severe mental deficiency precluded sufficient cooperation. Toilet training was not achieved. At the age of 20, she has no speech and very poor comprehension. Episodes of agitation and headbanging occurred during examination. Stereotyped movements with the hands and the body were also noticed.

In contrast, the normal twin was 162 cm tall; weight was 50.1 kg and she had no signs of BDLS. In addition, her mental development was normal and she graduated from high school with a good performance.

Chromosome analysis was performed on both sisters at the 600–1,000 band stage with G-banding. In particular chromosome 3 was specially scrutinized but no abnormalities were seen.

DNA was isolated from blood samples of the twin sisters as described elsewhere [Pena et al., 1991], digested with *HinfI,* run in electrophoresis, and blotted onto nylon membranes. The membranes were then hybridized with three multilocus probes: F10 [Pena et al., 1990, 1993], DNF24 [Ip et al., 1989] (Lifecodes Corporation, Stamford, CT), and 33.6 [Jeffreys et al., 1985] (Cellmark Diagnostics, Germantown, MD). All probes were oligonucleotides directly conjugated to alkaline phosphatase and were visualized by chemiluminescence after reaction with the substrate Lumiphos 480. With the F10 probe twenty bands were scored above 4 KB and 17 with 33.6, while with the DNF24 probe 5 bands were scored above 4 Kb and 17 with 33.6, while with the DNF24 probe 5 bands were scored in the whole gel. With all three probes there was absolute identity in the twin sisters. We can thus calculate that the odds in favor of the twins being MZ are 2×10^{27} to one (Lod score 27.3). Figure 2 shows the results with F10 and DNF24.

DISCUSSION

Although the BDLS has been known for almost 80 years, there is still lack of essential knowledge regarding the complete phenotypic spectrum and the natural history of this entity. The same is true regarding the cause and pathogenesis of this condition. In his recent editorial, Opitz [1994] emphasized that in spite of some progress achieved the BDLS still represents a continuing enigma and a challenge to us for the decades to come. The data of Feingold and Lin [1993] on some two dozen families, including four sets of concordant monozygotic twins is an attempt to review the literature on familial occurrences of BDLS and discuss information which suggests hereditary cause. They also contribute to this genetic data through their observation of a mildly affected mother and daughter. If inherited, the mildly affected cases are almost always transmitted by the mother. The overall most likely cause is an autoso-

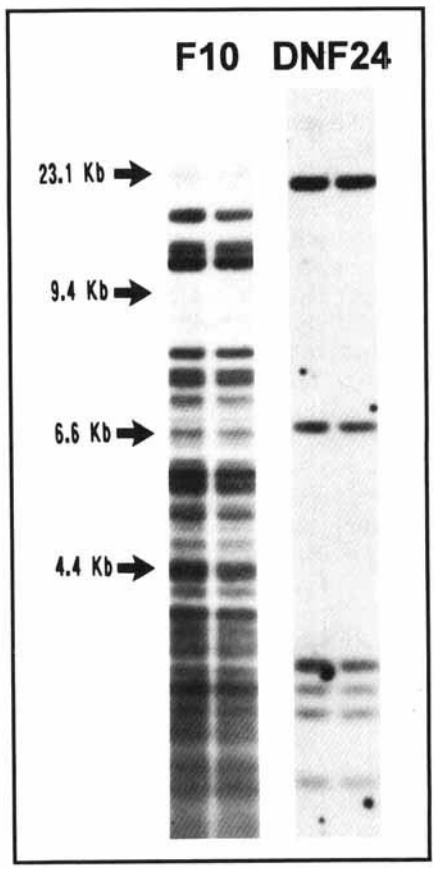

Fig. 2. DNA fingerprints of the two twins obtained by chemiluminescence with the multilocus probes F10 and DNF24. All probes were oligonucleotides directly conjugated with alkaline phosphatase. In the left, arrows indicate the migration of the *Hind*III digestion fragments of lambda phage, with molecular sizes, from top to bottom, 23.1 Kb, 9.4 Kb, 6.6 Kb, and 4.4 Kb.

mal dominant mutation with extremely variable expressivity that ranges from the mildness expressed in one parent to a prenatally lethal form of the disorder. The well-documented cases in the literature of familial recurrences (i.e., in sibs with apparently normal parents) can be explained on the basis of germline mosaicism for a new autosomal dominant mutation.

The search for a submicroscopic chromosome anomaly as cause of BDLS is still actively pursued, especially after the first reports of patients presenting with dup(3q) syndrome and some phenotypic similarity with BDLS [Wilson et al., 1978]. More recently, on the basis of a patient with a de novo translocation with breakpoints at 3q26.3 and 17q23.1, Ireland et al. [1991] proposed that a gene for BDLS was located at 3q26.3. Shaffer et al. [1993] tested 16 BDLS patients for uniparental disomy of chromosome 3 and did not find it in any case, although they could not rule out partial isodisomy of a segment on chromosome 3 not detected by their markers. Additionally, all of the probands studied by them demonstrated normal biparental inheritance for at least one locus, which argues against imprinting. De Marchi et al. [1994] have undertaken a similar study in 26 families and in 22 of these families none of the probands had uniparental disomy for chromosome 3. The other four families were uninformative for the markers used.

The reported concordance in four sets of MZ twins and discordance in three sets of dizygotic twins is perfectly compatible with the prevailing etiologic paradigm for BDLS, according to which most patients represent fresh mutations of an autosomal dominant gene. On the other hand, we here demonstrate unequivocally that the discordant twins originally described by Carakushansky and Berthier [1976] are indeed monozygotic. However there is no incompatibility if we assume that in the affected twin a somatic mutation occurred at around the time or shortly after the twinning event. This could occur by a submicroscopic chromosome deletion, a transposition event, the expansion of an unstable repeat, or a point mutation. Whatever the case, intensive comparative genomic studies of the discordant twin sisters here described should provide us with a unique opportunity to unravel the molecular genetics of this fascinating and enigmatic malformation syndrome.

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